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HISTOSTRUCTURAL APPRECIATION OF THE FORESTOMACH FIRST COMPARTMENT MUCOSA IN SHEEP

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Abstract

The mucosa of the ruminal wall was analyzed and measured in the different areas. First involved in this study was the ventral sac mucosa, and after were the pillars' region and the intermediary area between the reticulum and the rumen. Sheep from the white variety of the indigenous ovine breed Țurcană (Ovis aries) were used, the pieces of interest being collected and processed using conventional histological techniques, obtaining numerous seriated slides. After they were photographed and analyzed, we have been able to identify in the structure of the mucosa a cornified stratified squamous epithelium, lamina propria, and a densification of connective fibers. All three components of the mucosa form the ruminal papillae which reach the maximum height in the ventral sac area. We tried to classify them in organized groups, according to their average shape, length and width, by the thickness of the epithelium that lines each papilla, and the proportion occupied by the connective axis. In the area of the pillars, where the ruminal papillae are missing, the mucosa has the tendency to form extremely reduced folds, based on the thickening of the epithelium, that will subsequently attract the lamina propria. In the rumino-reticular junctional area, the papillae are reduced to the average length of 496 µm. The connective densification disappears, and in the deep layer of the mucosa, muscle fibers that detach from the superficial layer of the tunica muscularis and that will constitute the future papillary muscle, can be observed.

Key words: *sheep, histology, mucosa, papillae, ruminal wall.*

INTRODUCTION

The outstanding biological value, production efficiency and diversity of products made sheep very popular, as farmers have always sought to select and refine the ovine husbandry system.

In order to enhance the biological potential, selection, amelioration, as well as genetic exploitation of the structural diversity of local and imported breeds has been taken into account in configuring a precise, efficient and clear program of genetic improvement for each morpho-productive type. Thus, the lambs that resulted from crossing the Țurcană breed with other breeds specialized in meat production are well suited for fattening, and the adult sheep have the most diverse lines of operation, specialized for milk, meet, skin and wool production (Pascal, 1998 and 2002).

The economic results are directly influenced by the proper functioning of the digestive system and how the ingested forage is prepared for digestion, as the ruminal compartment has a very important role. The type of forage fed to the animals even in the first weeks of life will greatly influence the histostructure of this very important digestive compartment (Lane *et al*., 2000).

Studies concerning the volume of the ruminal digestion and degree of absorption, consistent with ruminal wall morphology focused on different races of large ruminants, in which a series of researches were carried out, in order to have morphometric (Melo *et al*., 2013) or structural (Graham and Simmons, 2005) assessments of the ruminal mucosa.

The climatic conditions in the northern European region, with great green masses and its digestion in game species have greatly influenced the morphology of the rumen papillae (Soveri and Nieminen, 2007).

Regardless of the ruminant specie, the histostructure of the rumen mucosa is characterized (Frandson *et al*., 2009) by the presence of papillae of different height, shape and diameter, with a marked tendency to cluster in certain areas of the organ (Tudor *et al*., 2005; König *et al*., 2007; Constantinescu and Schaller, 2012).

The whole rumen mucosa consists of stratified squamous keratinized epithelium (Cornilă,

2001), accompanied by *lamina propria* and a connective densification, sometimes mistaken for *muscularis mucosae* (Banks, 1993; Eurell and Frappier, 2006; Bacha and Bacha, 2012), which resulted in research showing a possible configuration of smooth muscle cells at this level (Ikemizu *et al*., 1994; Kitamura *et al*., 2003).

Therefore, this study analyzed and measured the mucosa of the rumen wall in the areas of the ventral sac, of the pillars and in the intermediary area between the reticulum and the rumen highlighting the histostructural peculiarities of the Ţurcană sheep breed.

MATERIALS AND METHODS

Sheep from the white variety of the indigenous ovine breed Ţurcană (*Ovis aries*) were used, initially represented by three seven-year-old adult females. Four more females of the same age were later added to the study, resulting in a total number of seven ovines used for this project, which is still ongoing. The sheep originated from an individual holding that owns a herd of approximately 200 of these ovines in a Subcarpathian area from Romania.

Slaughtering was done in a slaughterhouse which owns a specialized line for sheep, organized in accordance with the current European Community norms. Upon slaughter, sheep weighed between 45 - 48 kg.

The pieces of interest were collected and processed in the Histology laboratory of the Faculty of Veterinary Medicine of Bucharest using conventional histological techniques, obtaining numerous seriated slides stained with Hematoxylin and Eosin method and modified Mallory technique.

The examination of the histological slides was done using the Olympus CX42 optic microscope, and the images were captured with a Olympus E-330 photo camera and the quick CAMERA PHOTO 2.3 software, which allowed digital editing of images and a morphometric appreciation of the analyzed structures.

RESULTS AND DISCUSSIONS

In the structure of the mucosa, we could identify cornified stratified squamous epithelium, *lamina propria* and a densification of connective fibers. All three components of the mucosa form the ruminal papillae (Figure 1).

The basement membrane of the epithelium in the apical area of the ruminal papillae appears to be deformed by the compression imposed by the *lamina propria* in the shape of some thin, elongated, close and ordered folds that are permanently in contact with a capillary network (Figure 2).

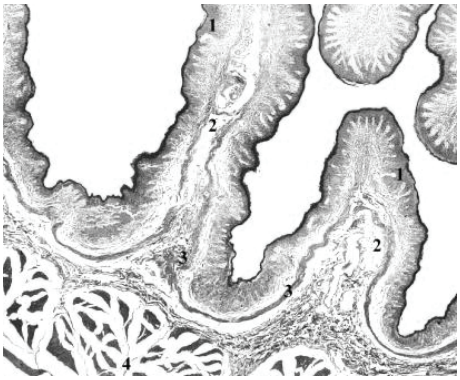

Figure 1. Photomicrograph of the ruminal wall, in the ventral sac area, showing straight papillae with wide, round apex/ Mallory stain, x40
1. Cornified stratified squamous epithelium;
2. Connective-vascular axis; 3. Connective densification;
4. External muscle layer.

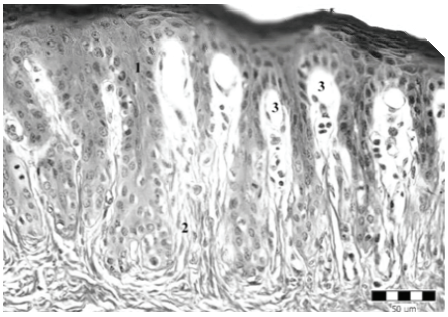

Figure 2. Epithelial detail of the apical papillary region/ Mallory stain, x400
1. Cornified stratified squamous epithelium;
2. *Lamina propria*; 3. Capillaries.

In the ventral sac area, the majority of the papillae reach the maximum height.

The studied sections reveal the presence of some papillae of different shapes and sizes that we tried to classify in organized groups, according to their average shape, length and width, by the thickness of the epithelium that covers them, and the proportion occupied by

the connective axis. The closer the papillae appear to be, the shorter and pointier they seem to be.

Tall papillae that have a relatively constant width are seen in the rumen mucosa of the ventral sac. Some are finger-like, straight and rounder towards the apex, like Constantinescu and Schaller (2012) observed generally in ruminants and not particularly in sheep. The average length is 2912 μm, the base has 695 μm diameter and the apex 641 μm. The connective axis is 422.4 μm wide, and the average thickness of the epithelium is 175 μm, varying between 58 and 294 μm.

Other papillae are extremely reduced, their length reaching only 331 μm, and they have a remotely conical aspect. The width of the base is 642 μm, of the tip 159 μm, and the average is 298.3 μm. The thickness of the epithelium is 74.5 μm, varying between 60 and 82 μm, and the connective axis is 452.8 μm, occupying mainly the area of the base of the papillae. In the case of these papillae, the connective densification does not extend into the papillae, but remains confined to the base. Also, curved papillae with oval shape, and an average height of 3039.5 μm have been observed in the ruminal wall. The connective axis and the epithetlium measure 1141 μm at the base, 1047.4 μm at mid-length, and 658 μm at the tip of the papillae. The papillary apex appears rounded. The interpapillary distance is 220 μm. These papillae have an intensely vascularized connective axis and the tendency to become curved and branched. Their apical epithelium forms regulated, thin, even, dense folds (Figure 3).

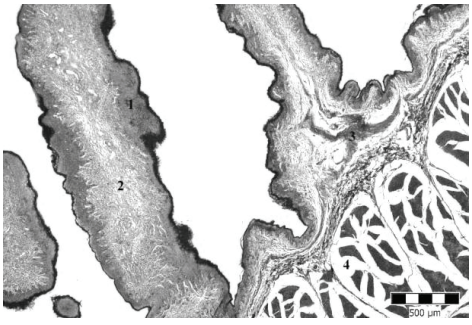

Figure 3. Reduced papillae, and finger-like papillae, straight (left) and thin (right), in the ventral sac area/ Mallory stain, x40
1. Cornified stratified squamous epithelium;
2. Connective-vascular axis; 3. Connective densification; 4. External muscle layer.

A series of rumen papillae have a narrowed basal region, of only 379 μm, giving an aspect of shrink structure. These papillae have an average length of 2215 μm and predominate in the ventral sac. The average width is 770.8 μm, with a widened tip measuring 910 μm and a base of 497.5 μm. The connective axis stretches interepithelially 484.3 μm, and the epithelium is 136.4 μm thick, varying between 66 and 213 μm (Figure 4).

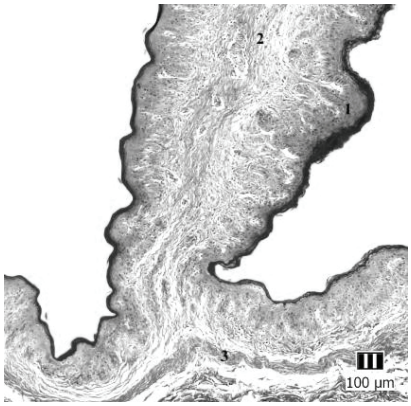

Figure 4. Photomicrograph of the ruminal wall, in the ventral sac area, showing papillae in the narrowed area/ Mallory stain, x40
1. Cornified stratified squamous epithelium;
2. Connective-vascular axis; 3. Connective densification;
4. Internal muscle layer.

The rarest papillae in the ventral sac were the branched ones. These have an average length of 3278 μm. The average width is 1312 μm, 619.5 μm towards the apex and 992 μm at the base. The connective axis has an interepithelial stretch of 827 μm, and the epithelium has an average thickness of 152 μm, varying between 67 and 238 μm.

In the structure of the rumen mucosa, the *muscularis mucosae* layer is absent (Ikemizu *et al*., 1994; Kitamura *et al*., 2003); instead there is a bundle of dense connective fibers (Figure 5).

In the area of the pillars, the rumen papillae are missing. The mucosa has the tendency to form extremely reduces pleats, based on the thickening of the epithelium, that will subsequently attract the *lamina propria.* The rumen mucosa has an average thickness of 321.5 μm, of which the epithelium makes up for 175.3 μm, and *lamina propria* for 104.5 μm. *Stratum corneum* is very thin, of 9 μm.

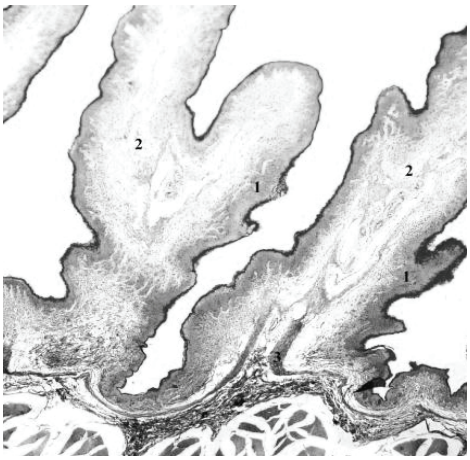

Figure 5. Photomicrograph of the ruminal wall showing branched papillae, in the ventral sac area/ Mallory stain, x40
1. Cornified stratified squamous epithelium;
2. Connective-vascular axis; 3. Connective densification;
4. Internal muscle layer.

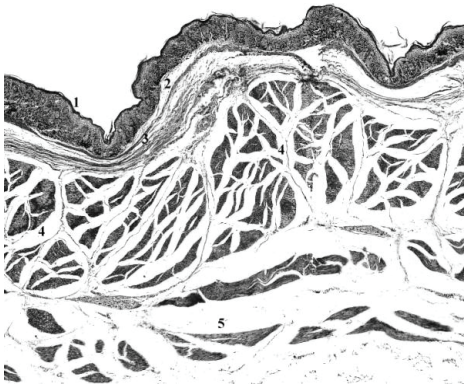

Figure 6. Histostructure of the ruminal wall in the pillars' area/ Haematoxylin-Eosin stain, x40
1. Cornified stratified squamous epithelium; 2. *Lamina propria*; 3. Connective densification;
4. Internal muscle layer; 5. External muscle layer.

It is made up of soft stratum corneum, showing nuclei of the superficial pavement cells in its structure. The connective densification is very thin, up to 41.7 μm and is very close to the epithelial basement membrane (Figures 6 and 7).

In the rumino-reticular junctional area, the papillae are reduced to the average length of 496 μm, only slightly taller than the inter-papillary mucosa, that reaches an average thickness of 447 μm, of which the epithelium is 218 μm, and *lamina propria* 229 μm. The stratum corneum reaches the average thickness of 27.7 μm.

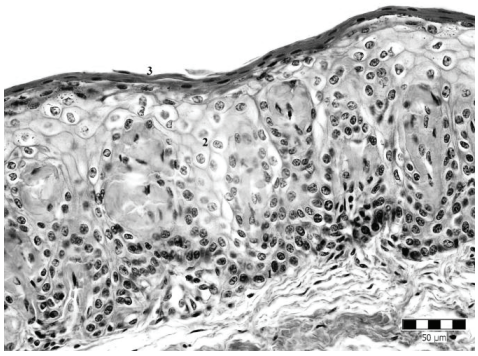

Figure 7. Histostructural detail of the ruminal mucosa in the pillar area/ Mallory stain, x400
1. Basal layer; 2. *Stratum spinosum*;
3. *Stratum corneum*.

The connective densification disappears, and in the deep layer of the mucosa, muscle fibers that detach from the superficial layer of the *tunica muscularis* and that will constitute the future papillary muscle, can be observed (Figure 8).

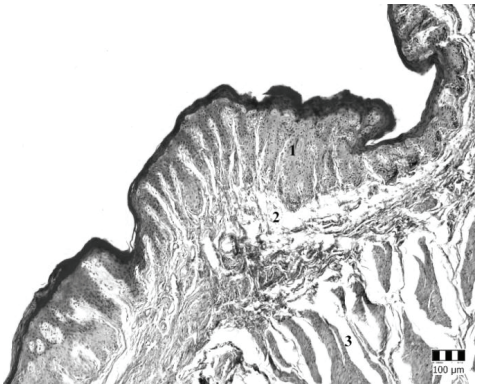

Figure 8. Histostructure of papillae in the ruminal wall, near the ruminal-reticular junction/
Mallory stain, x40
1. Cornified stratified squamous epithelium;
2. *Lamina propria*; 3. External muscle layer.

The morphometric analysis of the different structures in different macroscopic areas of the rumen wall revealed a series of characteristic elements. It may be noticed that the thickest mucosa (447 μm) is seen at the rumen-reticular fold, and the lowest value at the pillars (321.5 μm).

There repartition of the mucosal components differs by area. The average thickness of the epithelium and of the *lamina propria* is maximal in the mucosa of the rumino-reticular fold, but the thinnest epithelium is the one lining the ruminal papillae in the ventral sac

area. The *lamina propria* is least represented at the pillar mucosa (Table 1).

Table 1. Average morphometric values in different areas of the ruminal mucosa

Area of the ruminal wall	Mucosal thickness (µm)	Epithelial thickness (µm)	*Lamina propria* thickness (µm)	Connective densification thickness (µm)	Papillar length (µm)
Ventral sac area	335.8	126.3	142	67.5	2900
Pillars	321.5	175.3	104	41.7	-
Rumino-reticular fold	447	218	229	-	496

The whole rumen mucosa is sustained by a fibrous connective dense structure on its length (Ikemizu *et al*., 1994; Kitamura *et al*., 2003), except for the rumen-reticular junction, where fragmented connective bundles detach from the superficial area of the inner layer of the muscularis in order to structure on the *muscularis mucosa*, the future papillary muscle of the reticular wall (Figure 9).

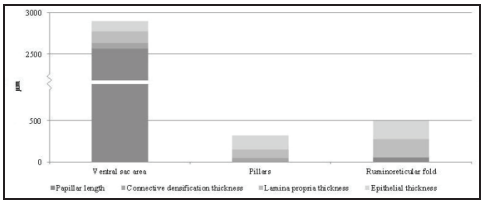

Figure 9. Proportional comparative representation of the ruminal mucosa components

The morphometric and histostructural analysis of the rumen papillae revealed significant differences in shape and size that allowed a classification of them into study groups. This sort of classification has not resulted so far out of specialized literature, neither for ruminants in general, nor for a particular breed of sheep. Neither Bacha and Bacha (2012) nor Soveri and Nieminen (2007) have not attempted to classify the structures of the ruminal mucosa, only writing a generalized view of them in sheep, and respectively in another ruminant, the forest reindeer (*Rangifer tarandus fennicus*).

The rumen papillae have a height that largely varies between 3278 µm and 331 µm, the tallest being the branched ones, and the others being the shortest papillae.

The structure that varies the least is the epithelium, which maintains its characteristics in all papillae.

The widest base ruminal papillae are both reduced, conical ones, and narrow, finger-like ones, the difference being of only 181µm.

The papillae most dilated in the apical are represented by the papillae with shrunken basis, and the narrowing ones are the reduced papillae. A correlation between the shrunken papillae and the interpapillary distance cannot be established. There are short, pointy papillae, where the papillae tend to be closer.

By analyzing the values given by the width of the connective axis and of the average width, we observed that the papillae with the widest base and the thickest connective axis have the greatest average thickness.

By individually measuring each papilla we have seen that the papillae with a length of 3000 µm predominate in the structure of the ruminal mucosa, while the short papillae of 331µm and the branched ones, of 3278 µm, are exceptions (Table 2, Figure 10). This average height is much larger than the average 1000 µm usually noticed in ruminants by Constantinescu and Schaller (2012).

Table 2. Average morphometric values for different papillae formed by the ruminal mucosa at the level of the ventral sac

Papillae	Average length (µm)	Average width middle area (µm)	Average width apex (µm)	Average width base (µm)	Epithelial thickness (µm)	Width of connective axis (µm)
Finger-like straight papillae	2912	691.8	641	695	175 (58-294)	422.4
Finger-like thin papillae	2457	498	412	1148	114.8 (79-157)	229.8
Short papillae	331	298.3	159	642	74.5 (60-82)	452.8
Conical papillae	1339.5	935.8	465.5	1329	119.5 (50-206)	886
Basally strangled papillae	2215	770.8	910	497.5	136.4 (66-213)	484.3
Branched papillae	3278	1312	619.5	992	152 (67-238)	827
Elongated oval papillae	3039.5	1047.4	658	1141	156.5 (60-250)	672.5

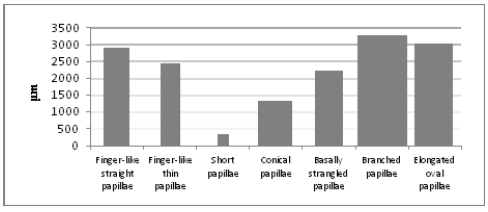

Figure 10. Morphometric appreciation of the ruminal papillae at the level of the ventral sac mucosa

CONCLUSIONS

The papillary epithelium retains its similar proportions throughout the ventral sac.

The most dilated papillae in the apical area were represented by basally narrowed ones.

The very pointed ones are actually the shortest in height.

A direct correlation between the basally narrowed papillae and the reduction of the interpapillary distance cannot be established: where the papillae tend to be closer, they appear shortened and pointier.

The thinnest epithelium is seen on the rumen papillae in the ventral sac.

The average thickness of the epithelium and of the *lamina propria* reaches the maximal value in the rumen-reticular fold area.

The fibers that will constitute into the papillary muscle of the reticular folds separate from the uppermost structure of the internal layer of the *muscularis externa* in the rumen-reticular fold area.

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